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(54) Title: METHODS FOR B-CELL POPULATION CONTROL

(57) Abstract

Methods are provided for inducing cell death in B-cells, including neoplastic B-cells, by employing reagents that bind to a B-cell epitope designated CDIM epitope. Particularly, antibodies specific for the B-cell marker can be administered to a host to induce death in B-cells to which the antibodies bind or can be used in *ex vivo* clinical situations to selectively remove B-cells. A B-cell specific oligosaccharide epitope useful as a B-cell marker has been identified. The ligand being recognized on B lymphocytes has no apparent similarities to any of the known pan-B cells markers. Agents which specifically bind the disclosed epitope and lead to B-cell death are provided. Provided is human monoclonal antibody 216, which recognizes this B-cell epitope and binds all $CD19^+$ and $CD20^+$ B lymphocytes in human peripheral blood and spleen, and is cytotoxic to B-cells. Furthermore, MAb 216 does not distinguish B cells by the isotype expressed, binding IgG^+ and IgM^+ cells with equal intensity, and also binds all B cells regardless of their CD5 expression. These products and methods find use in diagnosis and therapy.

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MEDLINE, BIOSIS, EMBASE, WEST 1.2 search terms: author names, cd20, bone marrow, transplant, stem cells, pbsc, c2b8, B cell, lymphoma

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,736,137 A (ANDERSON et al.) 07 April 1998, see entire document.	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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DETAILED DESCRIPTION OF THE INVENTION

A significant problem associated with the treatment of diseases involving B-cells and other cells that express CD20 antigen, including B-cell lymphomas and leukemias, is the problem of disease relapse after treatment.

The exact cause for disease relapse is unclear. However, it is known that such relapse may occur even in patients that receive aggressive therapeutic intervention, e.g., high dosages of chemotherapeutic agents, cytokines, radiation, and/or antibody. While the exact cause of relapse remains unclear, it is speculated by some researchers that disease relapse may occur because the patient may still harbor low numbers of diseased cells even after aggressive therapy. Also, it is speculated that bone marrow transplant or peripheral blood stem cell transplanted tissue may itself be contaminated by diseased cells that express the CD20 antigen, e.g., B-cell lymphoma cells. Therefore, transplant of such tissues may unwittingly introduce diseased cells, and thereby actually increase the risk of disease relapse.

As discussed, the present invention seeks to prevent or reduce the incidence of disease in patients receiving transplanted bone marrow or peripheral blood stem cells by treating the transplanted bone marrow or peripheral blood stem cells with an amount of an anti-CD20 antibody or fragment thereof effective to purge the transplanted tissue of disease-causing CD20 antigen-expressing cells. Such purging may be effected *in vitro* and/or *in vivo*.

For example, bone marrow or peripheral blood stem cells may be contacted in tissue culture with an anti-CD20 antibody prior to transplant. In the preferred embodiment such antibody will comprise a chimeric, primate, primatized®, humanized or human anti-CD20 antibody, preferably RITUXAN®.

Alternatively, or in conjunction with such *in vitro* purging, the patient may be treated concurrent or subsequent to bone marrow or peripheral blood stem cell

transplant with an amount of an anti-CD20 antibody effective to purge (*in vivo*) or at least reduce the number of disease causing cells that express CD20 antigen that may be present in the transplant.

Similarly, the antibody used for *in vivo* purging will preferably comprise a chimeric, humanized, primate, primatized®, or human anti-CD20 antibody, preferably RITUXAN®. This *in vivo* purging may be effected simultaneous or substantially contemporaneous to bone marrow or peripheral blood stem cell transplant. Preferably, such purging will be effected within a week or more, preferably within 1 to 12 hours after transplant. However, such purging can be effected up to about 1 to 100 days after transplant. In the preferred embodiment, *in vivo* purging will be effected within about 1 month after transplant, more preferably within about one week after transplant, and most preferably within about 1 to 12 hours after transplant.

As noted above, the subject *in vivo* or *in vitro* purging of CD20 antigen-expressing cells will desirably be effected in patients that have previously been treated in an effort to eradicate disease causing B-cells, or other CD20 antigen-expressing cells involved in disease. Such treatment methods include, by way of example, cytokine therapy, antibody therapies (e.g., RITUXAN® or other antibodies targeted to B-cells), chemotherapy and/or radiation therapy, e.g., whole body irradiation, radioimmunotherapy.

In an especially preferred embodiment, the subject *in vitro* or *in vivo* purging will be effected in patients that have previously been treated with RITUXAN® and/or radioimmunotherapy that receive an autologous bone marrow or peripheral blood stem cell transplant after RIT and/or RITUXAN® therapy.

For example, patients that have a B-cell-related disease, e.g., a B-cell lymphoma or leukemia, will have their bone marrow or peripheral blood stem cells collected prior to therapeutic treatment. This will be effected by known methods.

The patient will then be subjected to an aggressive therapeutic regimen, e.g., administration of RITUXAN®, or a radiolabeled antibody that is specific to an antigen expressed by the tumor cells, whole body irradiation, and/or a chemotherapeutic or cytokine. This therapeutic regimen will be effected under conditions that are hypothetically designed to eradicate any B-cell or other CD20 antigen-expressing tumor cells that may be present.

After such treatment has been completed, the bone marrow or peripheral blood stem cells, which optionally may be treated *in vitro* with an anti-CD20 antibody, e.g., RITUXAN®, to deplete CD20 expressing cells, is then transplanted into the patient in order to reconstitute the immune system thereof.

Concurrently or shortly thereafter, the patient will be administered an amount of an anti-CD20 antibody, e.g., RITUXAN®, effective to purge any disease causing cells that may be present in the bone marrow or peripheral blood stem cell transplant. An effective dosage will typically comprise from about 0.01 to about 3.0 mg/kg body weight. A preferred dosage will comprise from about .1 to about 20 mg/kg, more preferably from about .1 to about 5.0 mg/kg, administered within about one week of transplant.

The subject *in vitro* and/or *in vivo* purging will reduce the risk of relapse in many B-cell-related diseases, e.g., B-cell lymphomas and leukemias such as non-Hodgkin's lymphomas, chronic lymphocytic leukemia, etc., after treatment has been completed in patients receiving transplanted cells that potentially may be contaminated with disease-causing cells.

Also, the subject method should be well tolerated based on the relative non-toxicity of anti-CD20 antibodies, such as RITUXAN®, and therefore should not adversely impact engraftment of the transplanted autologous cells. In fact, it may act to promote engraftment of such transplant.

As noted in the preferred embodiment, the purging agent will comprise RITUXAN®. However, other anti-CD20 antibodies may be used, e.g., other chimeric, primate, primatized®, humanized or human antibodies. Also, antibody fragments may be used, e.g., Fv's, FAB, F(ab)', $F(ab_2)^1$, and aggregates thereof. In addition, antibodies and antibody fragments directed to other B cell surface markers, e.g., CD19, may also be used.

Methods for producing chimeric, primate, primatized®, humanized and human antibodies are well known in the art. See, e.g., U.S. Patent 5,530,101, issued to Queen et al, U.S. Patent 5,225,539, issued to Winter et al, U.S. Patents 4,816,397 and 4,816,567, issued to Boss et al, and Cabilly et al, respectively, all of which are incorporated by reference in their entirety.

The selection of human constant regions may be significant to the therapeutic efficacy of the subject anti-CD20 antibody. In the preferred embodiment, the subject anti-CD20 antibody will comprise human, gamma 1, or gamma 3 constant regions and, more preferably, human gamma 1 constant regions. The use of gamma 1 anti-CD20 antibodies as therapeutics is disclosed in U.S. Patent 5,500,362, issued to Robinson et al.

Methods for making human antibodies are also known and include, by way of example, production in SCID mice, and *in vitro* immunization.

As noted, a particularly preferred chimeric anti-CD20 antibody is RITUXAN®, which is a chimeric gamma 1 anti-human CD20 antibody. The complete amino acid and corresponding nucleic acid sequence for this antibody may

be found in U.S. Patent 5,736,137, which is incorporated by reference in its entirety. This antibody, which is produced in a proprietary CHO cell expression system commercialized by IDEC Pharmaceuticals Corporation, is made by a CHO cell transfectoma which was deposited on November 4, 1992, under the provisions of the Budapest Treaty at the American Type Culture Collection, located at 12301 Parklawn Drive, Rockville, MD 20852. This cell line was determined to be viable and will be replaced should it become non-viable during the term of deposit. This cell line was made irrevocably available upon issuance of the 5,736,137 patent and is available without restriction from the ATCC. This cell line will also be available without restriction during the lifetime of any patent that may issue based on this application.

The subject anti-CD20 antibody, when used as a purging agent, will be administered by various routes of administration, typically parenteral. This is intended to include intravenous, intramuscular, subcutaneous, rectal, vaginal, and administration with intravenous infusion being preferred.

The anti-CD20 antibody will be formulated for therapeutic usage by standard methods, e.g., by addition of pharmaceutically acceptable buffers, e.g., sterile saline, sterile buffered water, propylene glycol, and combinations thereof.

EXAMPLE

A single-arm pivotal study of Rituximab® infused at 375 mg/m^2 weekly times four was conducted in 166 patients with relapsed or refractory, low-grade or follicular NHL (International Working Formulation [IWF] Types A - D and REAL classification, small lymphocytic lymphoma, Follicular center, follicular Grades I, II, III). (McLaughlin P, Grillo-López A, Link B, Levy R, Czuczman M, Williams M, Heyman M, Bence-Bruckler I, White C, Cabanillas F, Jain V, Ho A, Lister J, Wey K, Shen D, Dallaire B. Rituximab® chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a 4-dose

treatment program. Journal of Clinical Oncology 1998; 16:2825-2833.) Patients with tumor masses > 10 cm or with > 5000 lymphocytes/μL in the peripheral blood were excluded from this study. The median age was 58 years (105 men and 61 women) and the median number of prior treatments was three. Bone marrow involvement was present in 56% of 149 patients evaluated. Forty-five percent had ≥ 2 extranodal sites and 41% had bulky disease (≥ 5 cm).

Complete response required the regression of all lymph nodes to $< 1 \times 1$ cm^2 demonstrated on two occasions at least 28 days apart on neck, chest, abdomen, and pelvic CT scans, resolution of all symptoms and signs of lymphoma, and normalization of bone marrow, liver, and spleen. Partial response required a ≥ 50% decrease in the sum of the products of perpendicular measurements of lesions without any evidence of progressive disease for at least 28 days. Patients who did not achieve a CR or PR were considered non-responders, even if a net decrease (> 50%) of measurable disease was observed. Time to progression was measured from the first infusion until progression.

The overall response rate (ORR) was 48% with a 6% CR and a 42% PR rate (McLaughlin P, Grillo-López A, Link B, Levy R, Czuczman M, Williams M, Heyman M, Bence-Bruckler I, White C, Cabanillas F, Jain V, Ho A, Lister J, Wey K, Shen D, Dallaire B. Rituximab® chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a 4-dose treatment program. Journal of Clinical Oncology 1998; 16:2825-2833). The median time to progression (TTP) for responders was 13.2 months and the median duration of response (DR) was 11.6 months. Twenty-two of 80 (28%) responders remain in ongoing remission at 20.9+ to 32.9+ months (McLaughlin P, Grillo-López A, Maloney D, Link B, Levy R, Czuczman M, Cabanillas F, Dallaire B, White C.

Efficacy controls in long-term follow-up of patients treated with rituximab for relapsed or refractory, low-grade or follicular NHL. *Blood* 1998; 92:414a-415a).

Administration of Rituximab® resulted in a rapid and sustained depletion of B-cells. Circulating B-cells were depleted within the first three doses with sustained depletion for up to six to nine months post-treatment in 83% of patients. Median B-cell levels returned to normal by 12 months following treatment. Although median NK cell counts remained unchanged, a positive correlation was observed between higher absolute NK cell counts at baseline and response to Rituximab® (Janakiraman N, McLaughlin P, White C, Maloney D, Shen D, Grillo-López A. Rituximab: Correlation between effector cells and clinical activity in NHL. *Blood* 1998; 92 (10 Suppl 1):337a).

Several baseline prognostic factors were analyzed to determine their correlation to response. Significantly, in 23 patients relapsed after ABMT or PBSC, the ORR was 78% versus 43% in patients who did not undergo prior high-dose therapy ($p < 0.01$). This suggests that anti-CD20 treatment may effectively be used to purge CD20 antigen-expressing cells *in vivo* when administered following transplantation. Moreover, because patients who receive prior high dose therapy accompanied a bone marrow or peripheral stem cell transplantation appear to benefit more from subsequent Rituximab® therapy than those patients without prior therapy and transplantation, this suggests that a combined treatment protocol including bone marrow or stem cell transplantation provides a synergistic effect when compared to either single treatment alone.

Although the present invention has been described in some detail by way of illustration and example, for purposes of clarity and understanding it will be apparent that certain changes and modifications may be practical within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for reducing the risk of relapse of a B-cell-related disease in a patient receiving a bone marrow or peripheral blood stem cell transplant comprising treating said transplant *in vitro* and/or *in vivo* with an amount of an anti-CD20 antibody effective to reduce (purge) the number of disease-causing CD20 antigen-expressing cells therein.

2. The method of Claim 1, wherein said disease is a B-cell lymphoma or leukemia.

3. The method of Claim 1, wherein said purging is effected *in vivo* by administering RITUXAN® no later than about one month after transplant.

4. The method of Claim 1, wherein RITUXAN® is administered at a dosage ranging from about 0.1 to 20 mg/kg about one week after transplant.

5. The method of Claim 4, wherein the patient receiving the transplant has previously been treated under conditions designed to eradicate disease-causing B-cells.

6. The method of Claim 4, wherein said patient has previously been subjected to a treatment protocol selected from the group consisting of whole body irradiation, RITUXAN® immunotherapy, chemotherapy, cytokine therapy, radioimmunotherapy, or a combination thereof.

METHODS FOR B-CELL POPULATION CONTROL

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of pending United States application serial number 08/101,436, filed August 2, 1993.

ACKNOWLEDGEMENTS

The research described in this application was supported at least in part by a grant from the National Institutes of Health. The government may have rights in any patent issuing on this application.

INTRODUCTION

Technical Field

The field of this invention is the control of B-cell proliferation in a mammalian host as a therapy.

Background

The immune system is the first line of defense against many pathologies. Particularly, the lymphoid compartment is concerned with monitoring tumorigenesis, invasion by pathogens, such as bacteria and viruses, aiding in the removal of foreign bodies, and the like. Essential to the ability of the lymphoid compartment to protect the host against the various pathologies is the ability to recognize self from non-self. In monitoring tumorigenesis, subtle distinctions may be involved and the high incidence of cancer, particularly in the aged, suggests that the monitoring frequently breaks down over time. In addition, because of the enormous diversity of the environment to which the immune system is exposed, there is always the possibility that epitopes will be encountered, which may trigger an immune response which can be directed against self. Other mechanisms may also be operative in the process where a lymphoid cell attacks an endogenous epitope. These autoimmune diseases

can be extremely destructive, as is evidenced by diabetes, rheumatoid arthritis, neuronal diseases, such as multiple sclerosis, and the like. While in many cases, the disease is associated with T-cell attack, in some of the diseases, there may be a B-cell component, and in other diseases, such as rheumatoid arthritis and lupus nephritis, the primary mediator may be B-cells.

The lymphoid compartment may be more susceptible than other cells to tumorigenesis, because of the recombinatorial processes associated with the rearrangements involved with formation of immunoglobulins and the T-cell receptor. Lymphoid cancers, such as lymphomas and leukemias are particularly dangerous, because of the opportunity for migration of the lymphoid cells throughout the body and the many sites in the periphery, where lymphocytes reside, so as to provide numerous opportunities for metastasis. Furthermore, these diseases interfere with the native process which is intended to monitor tumorigenesis.

There is, therefore, substantial interest in being able to develop techniques and therapies which will allow for selective reduction in cell types associated with pathogenesis.

Relevant Literature

Grillot-Courvalin et al. (1992) *Eur. J. Immunol. 22:1781*, describe an anti-B cell autoantibody from Wiskott-Aldrich syndrome which recognizes i blood group specificity on normal human B-cells. The production of human monoclonal antibodies is described by Bieber and Teng (1987) "*In vitro* sensitization for the production of human monoclonal antibodies," in *Human Hybridomas*, A.J. Strelkauskas ed. Marcel Dekker, Inc., New York, p. 39. Kannagi et al. (1983) *Cancer Res. 43:4997*, describe factors affecting expression of glycolipid tumor antigens. Niemann et al. (1978) *Biochem. Biophys. Res. Comm.* 81:1286, describe Blood group i and I activities of "lacto-N-nor-hexaosylceramide" and its analogues, particularly the structural requirements for i-specificities. Teng et al. ((1985) *Proc. Natl. Acad. Sci. USA* 82:1790) discloses the production of human monoclonal IgM antibodies against bacterial lipopolysaccharide ("LPS") and demonstrated that monoclonal antibody A6H4C5 was directed against covalently bound lipid A, the most conserved structure of LPS amongst Gram-negative bacteria.

SUMMARY OF THE INVENTION

Methods are provided for inducing cell death in B-cells, including neoplastic B-cells, by employing reagents that bind to a B-cell marker. Particularly, antibodies specific for the marker can be administered to a host to induce death in B-cells to which the antibodies bind or can be used in *ex vivo* clinical situations to selectively remove B-cells.

DESCRIPTION OF SPECIFIC EMBODIMENTS

In accordance with the subject invention, methods and compositions are provided for killing B-cells, particularly neoplastic B-cells, in cellular compositions comprising a plurality of cells, particularly hematopoietic cells. The method allows for a therapy in the treatment of the aberrant proliferation of B-cells and for the selective removal of B-cells from cultures or other *ex vivo* situations. By B-cells is intended those cells of the B-cell lineage, where B-cells may be defined as comprising surface membrane protein markers found on normal B-cells, such as CD19, CD20, CD21 and CD22.

The CDIM epitope is a three-dimensional structural conformation recognized on normal human, peripheral, and splenic B-cells and on some neoplastic B-cells by the human monoclonal antibody 216. The epitope is defined structurally in terms of spatial conformation, functionally in terms of specific antibody binding, and cytologically in terms of cellular distribution, as described below.

The spatial conformation of CDIM is characterized by being a hexa- or longer, straight-chain oligosaccharide comprising acyl-substituted repeating glucosamine subunits. The epitope is structurally related to the "I" and "i" antigens present on adult and cord red blood cells (RBC's) respectively. Structurally related synthetic sugars include lacto-N-norhexaosyl ceramide. The CDIM epitope can be identified on a B-cell surface using fluorescent-labeled human monoclonal antibody (MAb), particularly the human monoclonal antibody 216. Cells carrying the epitope can be analyzed, for example, by a fluorescence-activated cell sorter (FACS). For example, a human MAb can be biotin labeled and detected with fluorescent-labeled streptavidin, where control human MAbs do not bind to the human B-cells.

The CDIM epitope is naturally presented as a glyco-moiety found on substantially all peripheral B-lymphocytes and splenic B-lymphocytes and on certain cultured B-cell lymphoma lines, such as Lam, REH, and JY25. It is also found on 30-40% of primary B-cell lymphomas of various histopathologic classifications. At least about 90% of these various categories of cells, more usually about 100% of these cells, will present the indicated epitope. The human monoclonal antibody 216, which recognizes CDIM on B-cells as described above, does not recognize this epitope on cultured T-cells such as Peer and HUT 78, macrophage lines such as U937, since the CDIM epitope is not present on normal T cells, macrophages, NK cells, epithelial, endothelial or mesenchymal cells.

The present inventors first discovered that the CDIM epitope exists on B-cells, that certain human polyreactive natural MAbs can bind to the CDIM ligand on human B lymphocytes, and that the binding of at least two CDIM epitopes on the same B-cell leads to B-cell death. As demonstrated in the Examples section, human monoclonal antibodies designated MAb 216 and MAb A6H4C5 are among those that the have been discovered to bind to the CDIM epitope and lead to B-cell death.

The ligand recognized by 216 and A6H4C5 on human B lymphocytes is cleaved by the enzyme endo-β-galactosidase, indicating it is a carbohydrate antigen structurally related to the polylactosamine chain of i Ag. Grillot-Courvalin et al. ((1992) *Eur. J. Immunol.* 22:1781) have reported human MAbs (HY18 and HY21) derived from a single patient with Wiskott-Aldrich syndrome, which react with a human B cell subset and have cold agglutinin specificity for the i Ag of cord RBC. Unlike the HY18 and HY21 antibodies that react to only 60% of the $CD19^+$ cells in human tonsils, spleen, and peripheral blood, MAbs 216 and A6H4C5 bind all $CD19^+$ and $CD20^+$ B lymphocytes in human peripheral blood and spleen, MAbs 216 and A6H4C5 do not distinguish B cells by the isotope expressed, binding IgG^+ and IgM^+ cells with equal intensity, and also bind all B cells regardless of their CD5 expression. A6H4C5 and 216 also react to murine splenic and peritoneal $B220^+$ cells. The ligand recognized on B lymphocytes is apparently conserved among different species. It follows that the B-cell CDIM-binding molecule ("receptor") of the invention find use in diagnostics, particularly for methods that require B-cell detection, identification, number or percentage. Useful in diagnostics are kits that contain B-cell CDIM-

binding molecules of the invention. Using methods known in the art, the CDIM-binding molecules of the invention can be modified to contain detectable markers suitable for allowing detection of B-cell bound CDIM-binding molecules of the invention.

For the purpose of this invention polyvalent (two or more binding sites) CDIM-binding receptors are required. By "receptor" is intended a compound which has a specific affinity for the CDIM epitope, generally at least about 10^{-7} M, preferably at least about 10^{-8} M, so as to be able to bind the B-cell marker. The polyvalent nature of the receptor allows the simultaneous binding of at least two CDIM B-cell marker molecules on the cell membrane surface, thereby forming a cross-link. Conveniently, antibodies can be used from any of the immunoglobulin families, such as A, D, E, G, and M; it is not requisite that the antibody be associated with various cytotoxic processes associated with particularly Fc-initiated processes. Usually, the antibody will be IgM, since the pentameric structure of this molecule allows cross-linking unhindered by steric interference. Binding of at least two CDIM marker molecules on the same cell surface by the same receptor results ultimately in cell death. Besides antibodies, other receptors with the indicated affinity will find use, where the receptor can, for example, be associated with a lectin either naturally occurring or modified. Alternatively, small synthetic molecules can be devised which will allow for specific binding and cross linking of the CDIM epitope. In addition, one may subject the variable region of a MAb to mutagenesis to enhance the binding affinity of an antibody for the CDIM epitope, if desired. Combinatorial libraries, such as bacteriophage libraries displaying human Ig repertoires, employed with the teachings herein provide a diversity of polyvalent agents for screening to identify additional CDIM-binding agents. A portion of a CDIM-binding antibody of the invention that retains B-cell binding function can be conjugated to a cytotoxic molecule, such as a peptide toxin (e.g., pseudomonas exotoxin, ricin A chain), using methods known in the art to create new macromolecules capable of B-cell killing. A Fab portion of a MAb of the invention is preferred for conjugation. A particularly preferred conjugate is the fusion of the Mab 216 Fab2 portion with a pseudomonas exotoxin. Cytotoxic conjugates can be created using means known in the art including chemical conjugation, such as site-specific conjugation, and DNA gene fusion

technology, so long as the conjugate retains B-cell binding specificity and has B-cell toxicity.

Using the screening methods as taught herein other MAbs or polyvalent agents with ability to kill human B cells can be identified. Preferred antibodies for screening are those whose heavy chain region are encoded by the VH4.21 gene. MAbs for screening, from which MAbs of the invention are identified, can be obtained from hybridomas generated from B cells of hosts immunized with an antigen displaying the CDIM epitope or a structure with similar spatial conformation or from B cells stimulated with LPS or other cross-reactive antigen prior to fusion either by host immunization (Teng et al. 1985) or by *in vitro* sensitization of B-cells (see Examples; Bieber and Teng (1987) "*In vitro* sensitization for the production of human monoclonal antibodies," in *Human Hybridomas*, A.J. Strelkauskas ed. Marcel Dekker, Inc., New York, p. 39). Monoclonal antibodies useful in the invention can also be obtained by (1) fusing a heteromyeloma to EBV transformed B cells from normal peripheral blood or spleen, (2) selecting hybridomas that present antibodies that are IgM and 9G4 positive (9G4 is a rat anti idiotype Ab that is known in the art to specifically recognize VH4.21 protein chains in the framework region of an antibody (available commercially from F. K. Stevenson, Southampton University, S09 4XY, England)), (3) screening, by FACS for example, for monoclonals that bind to human B-cells, and (4) selecting those B-cell positives that bind to synthetic CDIM epitope as taught herein. All cytotoxic monoclonal antibodies are cold agglutinins with specificity for the i antigen of cord red blood cells, preferably "high titer" anti i, i.e. having an end point in the nanogram/ml range. The i antigen is the straight chain lactosamine as opposed to the branched chain of the I antigen.

The CDIM-binding agents can be used in therapy for treatment of B-cell proliferative diseases, such as B-cell neoplasia, systemic lupus erythematosus ("SLE"), and autoimmune diseases. Thus, the subject agents will find application in the treatment of autoimmune-mediated disease, particularly B-cell-mediated disease. And particular in rheumatoid arthritis and lupus nephritis. For example, the human MAb 216, by binding to at least two CDIM epitopes on the same cell, causes the death of the cell expressing this epitope. Epitope-mediated death does not require complement or cell-mediated lysis. While, for the most part, human cells in human patients will

be a primary interest, other animals, particularly domestic animals, will also be served by the subject methodology to the extent that a given agent reacts across species, which is readily determined by binding studies of the type described herein. It follows that injection into a host of an effective dose of B-cell cytotoxic molecules of the invention that kills all peritoneal B cells can be used to prepare B cell free compositions. This method is particularly advantageous for use with species where preparation of B-cell free compositions by conventional *in vitro* methods is time-consuming or inefficient. Mice are a preferred host for application of this method. MAbs 216 and A6H4C5 are preferred for use in this method.

For therapeutic uses, the compositions and selective agents disclosed herein can be administered by any convenient technique, which can vary depending on the nature of the compound/agent, the purpose and frequency of the treatment, and the like. For small molecular weight agents, oral administration is preferred, and enteric coatings are indicated where the compound is not expected to retain activity after exposure to the stomach environment. Generally the amount administered will be empirically determined, typically in the range of about 0.1 to 1000 μg active ingredient per kg of recipient, with adjustment by a physician or other person after consideration of clinical results. For administration of larger molecules such as antibodies, generally larger doses are needed typically in the range of about 1 to 10 mg per kg of host.

Large proteins are preferably administered parenterally or systemically, conveniently in a physiologically acceptable carrier, e.g., phosphate buffered saline, saline, or deionized water. Some agents such as antibodies can also be administered nasally. Typically, compositions are administered to a retained physiological fluid such as blood. Other additives can be included, such as stabilizers, or bactericides. These additives, if present, will be present in conventional amounts.

The subject agents can also be used for treating cell populations in culture to diminish the B-cell population, whether normal or neoplastic, in the culture. Thus, in mixed cultures, where one wishes to avoid interference by B-cells, where one is interested in studying antigen-presenting-cell mechanisms other than those associated with B-cells, where one is analyzing for cells associated with mediating secretion of a particular cytokine, or where one wishes to study a mixed cell population for other purposes without the presence of B-cells, this can be achieved by adding an amount of

the subject agent effective to remove substantially all of the B-cells present in the culture. In a similar manner *ex vivo* therapeutic treatments can be utilized in which blood is removed from a patient into an external environment (as in dialysis), treated to remove excess B-cells, and then returned to the patient. CDIM-binding molecules of the invention can be attached to solid surfaces for use in B-cell binding as a means to isolate or remove B-cells from a mixed cell population.

Where CDIM epitope-specific antibodies are administered therapeutically, it is desirable to minimize the likelihood of an immunogenic or allergenic response by using host-specific antibodies (e.g., human antibodies in humans). While intact antibodies are commonly used, the antibodies may be modified in a variety of ways, by enzymatic cleavage to provide fragments, reduction of disulfide linkages, and the like.

In referring to an isolated component or compound, the isolated component or compound will constitute at least about 1%, usually at least about 10%, and more usually at least about 50% by weight of the isolated material. By pure compound or composition is intended at least about 90%, usually at least 95%, and more usually at least about 99% by weight of the component or compound. Unless otherwise indicated, functional fragments will also be intended when referring to components or compounds.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Production, characterization, and conjugation of human MAbs.

Human MAb 216 was prepared by fusion of uninvolved spleen lymphocytes from a patient with nodular lymphoma. The cells were incubated *in vitro* with LPS and fused to the heteromyeloma line SHMD33. This antibody was found to be mu, lambda using peroxidase-labeled chain-specific antibodies (Cal Tag, South San Francisco, CA). Nucleotide analysis of the heavy chain V region revealed it was encoded by the VH 4.21 gene.

The human MAb A6H4C5 was prepared by fusion of the heteromyeloma SHMA6 with EBV-transformed lymphocytes from human spleen of a Hodgkin's disease patient immunized with the J5 mutant of *Escherichia coli* 0111-B4 (a mutant lacking the O-specific side chain) (Teng et al. 1985). The antibody was found to be μ κ by ELISA using peroxidase-labeled chain-specific antibodies (Cal Tag, South San Francisco). As discovered by the present inventors MAb A6H4C5 binds to human B-cells and leads to B-cell death.

Human MAbs were purified on high pressure liquid chromatography using a carboxymethyl column (BioRad, Richmond, CA). Hybridoma supernatant containing 1% FCS was diluted 1:4 with 20 mM Na acetate pH 5.5 The MAbs were eluted with 300 mM NaCl Tris buffer pH 8, dialyzed in PBS and concentrated if necessary on a Centriprep concentrator (Amicon, Danvers, MA). By PAGE analysis the purified material was 85-90% IgM and also contained transferrin and BSA. Concentration of the purified immunoglobulins was determined by sandwich ELISA using a human polyclonal IgM standard (Cooper Biomedical, Malvern, PA).

MAb 216 and other human IgM MAbs were biotinylated using N-hydroxysuccinimidobiotin (Pierce, Rockford, IL) at a ratio of 60 μg/mg IgM. A6H4C5 was biotinylated using biotin HPDP (N-{6-(biotinamido)hexyl}-3'-(2'-pyridyldithio) propionamide) (Pierce), a sulfhydryl reactive reagent following the manufacturer's protocol. Because A6H4C5 partially precipitates at pH $>$ 8, N-hydroxysuccinimidobiotin could not be used.

Using screening methods as taught herein other MAb with ability to kill human B cells can be identified. Additional MAb with ability to kill human B cells were identified including MAb TH, a $\mu\lambda$ Mab (derived from a B cell lymphoma; MAb obtained from F. Hsu of Stanford University) and MAb FS3, a $\mu\lambda$ MAb (derived from a patient with cold agglutinin disease; obtained from F. Stevenson, Southampton, UK). Sequence analysis revealed that all, including MAb 216 and A6H4C5, were VH4.21 and IgM.

Flow cytometry

Human adult splenic mononuclear cells were obtained from patients undergoing therapeutic splenectomy, and peripheral blood was obtained from normal volunteers.

Lymphoma cells were obtained by biopsy or laparotomy for removal of tumor. All procedures had the approval of the Committee for the Protection of Human Subjects at Stanford University. Spleens were gently teased apart in HBSS with 1% FCS and 0.2% DNase and passed through sterile nylon membranes to obtain single-cell suspensions. Peripheral blood and splenocytes were centrifuged at 800 g for 30 min through a ficol/hypaque gradient (Histopaque-1077, Sigma, St. Louis, MO). The mononuclear cell population was washed three times in HBSS with 1% FCS, and resuspended in staining medium (RPMI with 3% FCS, 1 mM EDTA, and 0.01 M HEPES)at 2.5 x 10^7 cells/mi.

Tumor tissue that had been removed from patients at surgery was disassociated into a cell suspension and frozen in DMSO with storage in liquid nitrogen. The cells were thawed and incubated overnight at 37 degrees before staining. The thawed cells were also incubated 24 hours with human MAb 216 or control human IgM MAbs and stained with propidium iodide (PI) which measures cell death.

Multi-parameter flow cytometric analysis (FACS) has been described in detail (Parks et al. (1986) *The Handbook of Experimental Immunology, supra*, p. 29). Fluorescent-labeled mouse MAbs against CD epitopes were from Becton Dickinson. 5 x 10^5 cells were suspended with predetermined saturating concentrations of each of the conjugated fluorescent antibodies in a final volume of 125 μl, and incubated on ice for 15 min. The cells were washed and resuspended in 200 μl of staining medium and analyzed on a highly modified dual-laser FACS II (Becton Dickinson, Mountain View, CA), interfaced with a VAX 6300 computer (Digital Equipment, Maynard, MA) running FACS/desk software (Moore and Kautz (1986) *The Handbook of Experimental Immunology. supra* p. 30). Dead cells are identified with the propidium iodide (1 μg/ml) signal collected in the APC- or TR-channel in experiments with three-colors (Parks et al. (1986) *supra*).

Endo-β-galactosidase treatment of cells

Peripheral blood B lymphocytes were incubated at 37°C for one hour at 5 x 10^6 cells/ml in Iscove's with 5% FCS, with 15 mU/ml of endo-β-galactosidase (Boehringer Mannheim, Indianapolis, IN). The cells were washed and stained for

FACS analysis. Cord RBCs at 50% concentration were incubated with 0.1 U/ml of endo-β-galactosidase at 37°C for 4 hours, washed and tested for hemagglutination.

RESULTS

216 MAb reacts with a carbohydrate ligand on human splenic and peripheral B lymphocytes.

Multiparameter FACS analysis of human mononuclear cells demonstrated that the MAb binds specifically to all B lymphocytes ($CD20^+$) obtained from human spleen and adult peripheral blood. MS2B6, a human monoclonal IgM used as an isotype control, did not bind human B lymphocytes, nor did other poly-reactive natural antibodies.

The B lymphocytes reacting with 216 were also positive for other pan-B cell markers, such as CD19, CD21, and CD22. Excess amount (10X) of antibodies to CD19, CD20, CD21, CD22, and IgM did not inhibit the binding of 216 to B cells. 216 does not distinguish between subsets of B lymphocytes, reacting with both $CD5^+$ and $CD5^-$ B cells. The MAb also did not distinguish between the isotope expressed, reacting with both surface IgG or IgM bearing B lymphocytes. *.

Mononuclear cells from human peripheral blood were treated with endo-β-galactosidase, and then stained with MAb 216. Reactivity to human B lymphocytes is significantly reduced in enzyme-treated cells. Expression of an unrelated B cell marker (CD19) does not change following enzyme treatment. Thus, sensitivity of both B lymphocytes and cord RBC to endo-β-galactosidase treatment, suggests that the epitope recognized by the two antibody on B lymphocytes is also a carbohydrate antigen similar to the linear polylactosamine structure of the "i" antigen.

216 MAb binds to lymphoma cells

Twenty-seven primary lymphomas were analyzed by FACS. Twenty-three were B-cell lymphomas. The MAb 216 did not react with any T-cell lymphomas and stained 10 of 23 B-cell lymphoma. The MAb 216 did not stain any small cleaved cell (follicular) lymphoma. The MAb 216 stained the following classes of lymphoma;

immunoblastic, diffuse large well differentiated, diffuse large cell, diffuse mixed, and diffuse small cell.

In vitro B-cell toxicity of 216

Human MAb 216 is incubated in vitro at an Ab concentration of 10-20 μl/ml with various types of cells. The cells are in tissue culture media with heat-inactivated normal human serum or in serum-free media and are incubated at 37°C in 5% $C0_2$. After 18-24 hours incubation the cells are stained with propidium iodide (PI) (and other Ab for determining type of cell) and analyzed on FACS. Cell death is determined by uptake of PI. When human spleen or peripheral blood lymphocytes are incubated with MAb 216, 60-80% of B cells are killed. Four primary lymphoma cell suspensions that stained with the MAb 216, and two that did not, were incubated 24 hours with either 20 μg/ml of the 216 MAb or control human MAb in medial at 37 degrees in 5% CO_2. The cells were analyzed for cell death using propidium iodide (PI) on FACS. The lymphoma cell suspensions that bound the MAb 216 showed significant PI uptake compared to the control MAb. The two lymphoma cell suspensions that did not bind MAb 216 did not take up PI. When B cell lymphoma lines are incubated with MAb 216, 60-90% of the cells are killed. MAb 216 does not kill T cells, NK cells or monocytes. Other control human MAbs cause 0-5% cell death under the same conditions.

MAbs were incubated in vitro in serum free media with human spleen lymphocytes or B-cell lymphoma lines, and dead cells were determined by FACS analysis with PI. (Cell lines treated with 216 were sorted for PI positive and negative cells on FACS to confirm that the PI+ cells were dead.) Killing was maximal at 16-18 hours and 30-40 μg/ml MAb 216 for $3x10^6$ spleen lymphocytes. Using spleen B cells 70-90% of B cells were killed. Variation in percent killed was observed to vary with the individual spleen. MAb TH and MAb A6H4C5 killed the same percent of B cells at the same concentration of antibody as MAb 216. MAb FS3 killed one-fourth to one-third less cells at the same concentration as 216. MAbs A6H4C5 and TH killed B-cell lymphoma cell (4 different lymphoma cell lines were assayed) with the same efficiency as MAb 216; Mab FS3 was less efficient. In the presence of human complement *in vitro* all four MAbs killed 90-100% of B-cells.

Isolation of a B-cell Marker Molecule Having the CDIM Epitope

The B cell membrane glycolipids, proteins and glycoproteins, including the B-cell marker molecule having the CDIM epitope, can be extracted from the B cell membrane using techniques known in the art such as solubilization using a non-ionic detergent (such as Triton X). The B-cell membrane solution can be passed over an affinity matrix, such as a column, to which the MAb 216 has been covalently bound, for example, by using N-hydroxy succinimide coupled to acrylamide beads (Affi Gel). The CDIM epitope will bind to the MAb 216 resulting in the B-cell marker molecule having the CDIM epitope being removed from the membrane solution. The column is then washed to remove non-specifically bound proteins or molecules. The B-cell marker molecule having the CDIM epitope can be released from the column by competition with a compound such as lacto-N-nor-hexaosylceramide, contained in liposomes, which will competitive bind to the MAb 216.

Characterization of CDIM Antigen

The MAb 216 binds to synthetic I/i antigen.

The following glycolipids were run on TLC: i antigen (lacto-N-norhexaosylceramide), sialyl i antigen, paragloboside (lacto-N-tetraocylceramide), sialyl paragloboside, I antigen (branched), and GM3. The MAb 216 was applied to the plate. The plate was washed and I^{125} -labeled goat anti-human IgM was applied to the plate, washed and incubated with X ray film. The TLC plate was then sprayed with sulfuric acid and heated to visualize the glycolipids. Comparing the TLC plate and exposed film revealed that 216 bound i antigen, sialyl i antigen, I antigen, and sialyl I antigen only. i Antigen (Lacto-N-nor-hexaosylceramide): Galβ 1-4 GlcNAcβ 1-3 Galβ 1-4 GlcNAcβ 1-3 Galβ 1-4 Glcβ 1-Ceramide. Sialyl I Antigen: NeuAcα 2-3 Galβ 1-4 GlcNAcβ 1-3 Galβ 1-4 GlcNAcβ 1-3 Galβ 1-4 Glcβ 1-Ceramide. Paragloboside (Lacto-N-tetraocylceramide): Galβ 1-4 GlcNAcβ 1-3 Galβ 1-4 Glcβ 1-Ceramide. Sialyl Paragloboside: NeuAc α 2-3 Galβ 1-4 GlcNAcβ 1-3 Galβ 1-4 Glcβ 1- Ceramide. GM3: NeuAc α 2-3 Galβ 1-4 Glcβ 1-Ceramide.

In Vivo B-Cell Killing

Twenty μg of either Mab 216 or A6H4C5 when injected IP in normal mice killed all the peritoneal B cells within 18 hours. A B-cell free composition was obtained from the injected mice.

In Vivo Inhibition of Neoplastic B Cell Growth

The *in vivo* inhibition of growth of the human neoplastic B cell line LM3M by an antibody of the invention was demonstrated using death as an endpoint in Balb SCID ("severe combined immunodeficiency") mice. SCID mice, challenged with lymphoma cell lines, have been used extensively to test therapies for lymphoma, particularly human lymphoma. $1x10^5$ LM3M tumor cells were injected intraperitoneally (IP) into SCID mice. Three days after lymphoma cell line injection six test mice received 100 μg MAb 216 IP using a 25 gauge needle in 300 μl of solution, and six control mice received 100 μg MAb MS2B6 (a human IgM monoclonal antibody used as a control) IP using the same conditions as for the test mice. MAb MS2B6 is a human IgM lambda monoclonal antibody, which reacts with the intracellular nuclear matrix, and does not react with cell membrane or kill cells. Mice were observed daily until death. The amount of MAb injected in the mice was equivalent on a weight basis to a 70 kg human receiving 350 milligrams of MAb.

Mice receiving MAb 216 died 46, 53, 55, 55, 62, and 62 days after injection of the human LM3M tumor cells. The mouse that died after 46 days had an abnormal presentation displayed as a small (0.5 cm) tumor located on the outside abdominal wall. The mean survival time of mice receiving MAb 216 was 55.5 $\pm$ 6 days and was 57.4 $\pm$ 4.2 when the mouse with the abnormal presentation was excluded. Mice receiving control MAb MS2B6 died 45, 46, 49, 49, 49, and 52 days after injection of the human LM3M tumor cells. The mean survival time of mice receiving MAb MS2B6 was 48.3 $\pm$ 2.5 days. Survival times were significantly different as analyzed for significance using the t test. Degrees of freedom were 5 and the probability of significance was .0038 ($P > .005$). Administration of MAb 216 inhibits the growth of B cell lymphoma as indicated by the delayed onset of death in the mice treated with MAb 216. Even a single dose of a B-cell cytotoxic antibody resulted in a significant increase in survival times.

SUMMARY

216 binds all $CD19^+$ and $CD20^+$ B lymphocytes in human peripheral blood and spleen. Furthermore, 216 does not distinguish B cells by the isotype expressed, binding IgG^+ and IgM^+ cells with equal intensity, and also bind all B cells regardless of their CD5 expression. Accordingly, the ligand being recognized on B lymphocytes is a novel marker, with no apparent similarities to any of the known pan-B cells markers.

It is evident from the above results and disclosure that a novel B-cell marker and a specific oligosaccharide epitope thereof have been identified. In addition, proteins which specifically bind the disclosed epitope are provided. These products and products derivable therefrom find use in diagnosis and therapy.

A deposit of the hybridoma cell line secreting MAb 216 was made with the ATCC in Rockville, Maryland, USA, as Deposit No. HB 11659, on June 14, 1994.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A monoclonal antibody, wherein said monoclonal antibody recognizes a CDIM epitope and at least 90% of all B cells.

2. The monoclonal antibody of Claim 1, wherein said monoclonal antibody is an IgM.

3. A method for killing B cells within a mixed population of cells, said method comprising:

contacting said mixed population of cells with a cytotoxic amount of a polyvalent agent that binds more than one CDIM epitope.

4. A method according to Claim 3, wherein said polyvalent agent is an antibody.

5. A method according to Claim 4, wherein said antibody is a monoclonal antibody.

6. A method according to Claim 5, wherein said monoclonal antibody is a human antibody.

7. A method according to Claim 5, wherein said monoclonal antibody is an IgM.

8. A method according to Claim 3, wherein said B cells are neoplastic.

9. A method according to Claim 3, wherein said mixed population of cells is hematopoietic.

10. A method according to Claim 3, wherein said mixed population of cells is within a mammalian host.

11. A method according to Claim 10, wherein said B cells are neoplastic.

12. A method according to Claim 10, wherein said B cells mediate an autoimmune disease.

13. A method according to Claim 10, wherein said agent is a monoclonal antibody.

14. A method according to Claim 13, wherein said antibody is a human IgM.

15. A method of isolating a B-cell marker molecule, wherein the marker is characterized by having a CDIM epitope, is surface accessible on B cells, and is recognized by MAb 216, said method comprising:

combining a lysate, made from a cell population containing B cells, with an antibody binding to the CDIM epitope to form an immune complex;

separating said immune complex from other components of said lysate; and

releasing the B-cell marker molecule from said complex with a compound competitive for binding to said antibody.

16. A method according to Claim 15, wherein said competitive binding compound is lacto-N-norhexaosylceramide.

17. The monoclonal antibody of Claim 2, wherein the monoclonal antibody is the monoclonal antibody secreted by hybridoma cell line ATCC deposit No. HB 11659.

18. The hybridoma cell line ATCC deposit No. HB 11659.

19. The method of Claim 6, wherein the monoclonal antibody is the monoclonal antibody secreted by hybridoma cell line ATCC deposit No. HB 11659.

20. A diagnostic kit comprising the monoclonal antibody secreted by hybridoma cell line ATCC deposit No. HB 11659.

21. A kit for use in the method of claim 3, said kit comprising a cytotoxic amount of a polyvalent agent that binds more than one CDIM epitope.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08793

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	THE JOURNAL OF IMMUNOLOGY, VOL. 151, NO. 9, ISSUED 01 NOVEMBER 1993, N.M BHAT ET AL., "HUMAN ANTILIPID A MONOCLONAL ANTIBODIES BIND TO HUMAN B-CELLS AND THE i ANTIGEN ON CORD RED BLOOD CELLS", PAGES 5011-5021, ENTIRE DOCUMENT.	1-21
Y	PROC. NATL. ACAD. SCI. USA, VOL. 82, ISSUED 19 MARCH 1985, N.N.H. TENG ET AL., "PROTECTION AGAINST GRAM-NEGATIVE BACTEREMIA AND ENDOTOXEMIA WITH HUMAN MONOCLONAL IGM ANTIBODIES", PAGES 1790-1794, ENTIRE DOCUMENT.	1-21
Y	US, A, 4,804,626 (BELLET ET AL.) 14 FEBRUARY 1989, COLUMN 8, LINE 64 THROUGH COLUMN 9, LINE 9.	20-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 SEPTEMBER 1994	25 NOV 1994

Name and mailing address of the ISA/US	Authorized officer
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08793

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,904,481 (FATHMAN) 27 FEBRUARY 1990, COLUMN 10, KITS.	20-21
Y	J.W. GODING, "MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE", PUBLISHED 1986 BY ACADEMIC PRESS (LONDON) , PAGES 219-240, SEE ENTIRE DOCUMENT.	15-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08793

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61J 1/00; A61K 38/16, 39/00; C07K 16/30, 1/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/413, 388.15, 388.73; 424/179.1; 435/70.21, 172.2, 240.27

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

530/413, 388.15, 388.73; 424/179.1; 435/70.21, 172.2, 240.27

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, BIOSYS, EMBASE, LIFESCI